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Antioxidant And Antimicrobial Activity Of Silver Nanoparticles Biosynthesized Using *Crataeva Nurvala*buch. Ham. Leaf Extract

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ABSTRACT: Nano-technology has newlyoccurred as a fastrising field with many bio-medical science applications. By the time, silver has been accepted as an anti-microbial material showingfree radical scavenging activity that is relatively free of adverse effects. Therefore, this research has been carried out to estimate the anti-microbial and anti-oxidant activity of *Crataevanurvala*leaf extract and silver nanoparticle (AgNPs) synthesized by *Crataevanurvala*leaf (CNL-AgNPs). The characterization of AgNPs was done by using by using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The anti-microbial activity of CNL-AgNPsand methanol extract of *C. nurvala*leaf (CNLM) were tested against bacterial strains such as *P. aeruginosa, S. aureus, E. coli*,and*B. subtilis*and fungal strains like *C. albicans, T. reesei, A. niger,* and *P. chrysogenum* using the well diffusion technique. The prominent transmission band in FTIR spectra was observed at 3435 cm^{-1} (>N-H-), 1689 cm^{-1} (Quinone or conjugated ketone) and 1083 cm^{-1} (Si-O-Si). The range of particle size in SEM was between 29-82 nmwith spherical shape. CNL-AgNPs showed more diameter of inhibition against *Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis*with an inhibition zone of 16mm, 12mm, and 15 mm respectively when compared with CNLM. CNL-AgNPsexhibited prominent antifungal properties against *Candida albicans, Aspergillus niger*with 16mm inhibition diameter. Additionally the highest catalase (0.062 µM H_2O_2 reduce/gm Fwt/sec), peroxidase (0.558 µM/L/gm dwt/sec) and FRAP (1.799 µM at 100 µl) antioxidant activity were also observed with synthesized AgNPs in comparison to CNLM. Overall, the results concluded that the synthesized silver nanoparticle of *C. nurvala* leaf was more effective as antimicrobial and antioxidant agents as comparedto *C. nurvala*leaf.

Keywords: Nanotechnology, *Crataevanurvala*, Silver nanoparticle (AgNPs), Fourier transform infrared spectroscopy, Scanning electron microscopy, FRAP, Antimicrobial activity, Antioxidant activity.

INTRODUCTION

Since the foundation of humanoid, plant derived compounds and plants have been applied in numerousillnessesall over the world. From time immemorial plants has been utilized directly or indirectly as a source of drug. About 25 percent of the medicinesgiventhroughout the worldhave been derivedfrom plants.One of crucial ethnopharmacological plants that is used to cure a variety of illnesses is the *Crataeva nurvala* Buch. Ham. (Family Capparidaceae). (Hade *et al.,* 2016).The *Crataeva* genus mostly consists of roughly 70 species that are widely scattered over the world's

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warmer regions. The most diverse species in India is C. nurvala, which stands out among them (Bhattacharjee*et al.,* 2012).The three-leaved caper, *Crataeva nurvala* (also known as*C. roxburghii, C. magna*, and *C. religiose)*, is a popular name for this plant(Anonymous, 2004; Nadkarni and Nadkarn, 2009; Remya*et al.,* 2009).

In recent years, interest in nanotechnology has grown dramatically on a global scale.A nanoscale element with a size in the range of 1 and 100 nm is referred to as a nanoparticle. Due to their distinct physical, biological, and chemical characteristics, silver nanoparticles (AgNPs), among the metallic nanoparticles, have drawn more and more interest. AgNPs are extensively utilised as anti-bacterial (Franci*et al.,* 2015), anti-viral properties (Bekele *et al.,* 2016), anti-fungal (Medda*et al.,* 2015), and anti-inflammatory (Hebeish*et al.,* 2014). AgNP synthesis using plant extracts orbiological microorganisms has become a popular and straightforward substitute over chemical synthesis.The green synthesis process offers improvements over chemical approaches since it is economical and environmentally benign.When compared to other biological procedures, the synthesis of AgNPs via plant extracts might be beneficial since it doesn't need the upkeep of aseptic condition and cell cultures (Loo *et al.,* 2012). Consequently, a number of experiments employing plant extracts to create AgNPs in a greener manner have been described (Medda*et al.,* 2015; Dhand*et al.,* 2016;Ahmed *et al.,* 2016; Selvam*et al.,* 2017).

Typically, reducing metal cations with living being such as extracts of plants, or inactivated tissue, lichens, bacteria, yeast, fungus, and algaeresult in the creation of nanoparticles.In compared to nanoparticles produced chemically, those produced by bio-synthesis is not much harmful and is more constant.The component of bio-active extract's, like carotenoids,carbohydrates, biological catalysts (enzymes), trace metal ions,polyphenols,proteins,alkaloids,fats, among others, are crucial for the development of nanoparticlessince they act as effective reducing agents, stabilizers, or pre-cursor molecules for NPs (Martínez-Cabanas *et al.,* 2021; Zaharescu and Blanco, 2021; Alharthi*et al.,* 2021). Moreover, their capacity to makesilver nanoparticles, natural extracts have strong antioxidant activity that is greater than that of substrates as shown in the studies done before.The preferred absorption of extract components on the outwardsurface of nano-particles is thought to be the cause of this action (Patil and Kumbhar, 2017; Khandel*et al.,* 2018). It was observed that the polyphenols found in the plant extracts are responsible for the antioxidant activity displayed by metallic nanoparticles.Since they either give up their e^- or their H atoms, poly-phenols are strong anti-oxidants that may counteract free radicals.Inhibition of free radical precursors or deactivation of active species prevents the production of free radicals (Parashant*et al.,* 2015). Compared to other nanoparticles, silver nanoparticles have significantly greater antioxidant activity (Ramamurthy *et al.,* 2012). Consequently, they have positive impacts on human health and can provide oxidative stress protection (Prakash *et al*., 2011). Although *C. nurvala* has demonstrated success in treating a variety of diseases, but no comprehensive research on the synthesis of silver nanoparticles (AgNPs) using *C. nurvala* leaf extract has been published to yet. In order to identify the *Crataeva nurvala* leaf extract's best antibacterial and antioxidant properties, *Crataeva nurvala* leaf silver nanoparticles were made.

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MATERIAL AND METHOD

1. COLLECTION OF PLANT MATERIALS

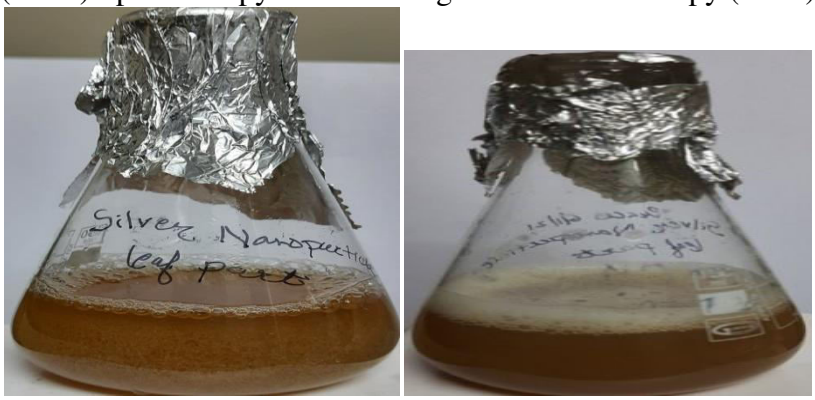
The experimental plant material of *Crataevanurvala* leaf was collected from near Albert Hall in front of Zoo, and adjoins area of Jaipur, Rajasthan.

2. PREPARATION OF PLANT EXTRACTS

The freshly harvested leaf section of *C. nurvala* is properly cleaned with tap water before being let to air dry for around two to three weeks at room temperature (32 to 37°C).Using a homogenizer, the dried plant samples were reduced to powder. In a Soxhlet extractor, 50g of powdered plant material (50g/250ml) were consecutively extracted with methanol for 8 to 10 hours. After obtaining the extracts, they were concentrated, and then dried to a consistent weight. Prior to conducting additional testing, dried extracts were stored at 20°C.

3. PREPARATION OF SILVER NANOPARTICLES

Using *C. nurvala*leaf extracts as a starting point, the Loo *et al*., 2012method was used to create AgNPs (CNL-AgNPs). 100 ml of distilled water and 10 grams of *C. nurvala*leaf powder were added to a beaker, which was then boiled at 60°C for 10 minutes. After 10 minutes, a 0.45 µm Millipore membrane filter was used to filter the leaf extract, and then a 0.2 µm Millipore membrane filter was used. $AgNO_3$ (1 mM) was dissolved in 100 ml of Erlenmeyer flask filled with 12 ml of leaf extracts to create AgNPs. There were visible variations in the solution's colour. Characterization of silver nanoparticle was done by using Fourier Transform Infrared (FTIR) Spectroscopy and Scanning electron microscopy (SEM).



(a) **(b)**

Figure 1: Picture of *Crataevanurvala* leaf extract with AgNPs solution (a) before and (b) after the CNL-AgNPs

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4. CHARACTERIZATION OF SILVER NANO-PARTICLES

Scanning electron microscopy (SEM)

Using a SEM [Carl ZEISS EVOR-18, Germany] operated at an extra high tension or accelerating voltage [EHT] of 20 kV, where WD was 8.5 mm, the real sizes of CNL-AgNPs were investigated. The sample discs were gradually loaded with a little quantity of the test ingredients. Before placing the materials on the specimen stage, the Quorum Q150RS rotary pumped sputter coater applied sputter coating (gold coating) to the materials for improved SEM image.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Shimadzu Spectrometer [IR Affinity-1; class-1 laser product, Japan] diffuse reflectance operating mode with a resolution of 4 cm1 was used to examine the sample. This device needs a tiny quantity of dried, KBr pellet-ground material. This activity was carried out to learn about the functional groups that are spread over the material surfaces.

5. ANTIMICROBIAL ACTIVITY

Microbial Strains, culture medium and inoculum preparation:

Clinical laboratory bacterial strains of *Bacillus subtilis* (MTCC 10619),*Pseudomonas aeruginosa* (MTCC 0424), *Escherichia coli* (MTCC 443) and*Staphylococcus aureus* (MTCC 3381)and fungal strain viz. *Candida albicans*(MTCC 183) , *Aspergillus niger*(MTCC 872), *Trichoderma reesei*(MTCC 164) and *Penicilliumchrysogenum*(MTCC 5108)were collected from the standard cultures of Microbiology Laboratory, SMS Medical College Jaipur, Rajasthan.

Determination of Antibacterial Assay

The agar well diffusion technique was used to inspect the CNL-AgNPs and CNLM*in vitro* anti-bacterial efficacy in contrast to bacterial strains (Gram negative and Gram positive) (Perez *et al*., 1990). The bacteriological medium was Mueller Hinton agar No. 2 (Hi Media, India). In 100% Dimethylsulphoxide (DMSO), the methanol extract were diluted to a concentration of 10 mg/ml. To create a solid plate, Mueller Hinton agar was liquefied, chilled, and then put onto sterile petri plates. It was made using a standardized inoculum (1.5108 CFU/mL, 0.5 McFarland) and sterile 0.9% saline water.The seeded agar plates were prepared with 6 mm wells. The test substance was added to the well in increments of 20, 40, 60, and 80 µl. At 37°C, the plates were incubated over night. Zone diameters surrounding each well were used to assess the extract's antibacterial spectrum for each type of bacterial species. The agent's zone of inhibition diameters were compared to those of the commercially available control antibiotic, Ciprofloxacin (40 µl).In order to measure the resultant zone diameter using an antibiotic zone reader to the closest mm, the antibacterial drug were deducted from the test zones. To reduce error, the experiment was run three times; the mean data are shown.

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Determination of Antifungal Assay

Well diffusion method was used to study the CNL-AgNPs and CNLM anti-fungal activity (Bonjar*et al.,* 2005). The fungi were revive onto SDA (Merck, Germany) and incubated for 24h at 37° C and 25°C for 2 to 5 days, respectively. The concentration of the fungal spore suspensions in sterile PBS was set at 106 cells/ml. rolling on the agar medium's surface after dipping a sterile brush into the fungus solution. The plates were dried for 15 min. at RT. Using a decontaminated glass tube, 6 mm-diameter holes were made in the culture medium. For each well, 20, 40, 60, and 80 µl of fresh extracts were given until full.At 37°C, plates were incubated. Bio-activities were measured by measuring the width of the inhibitory zone after a 24-hour incubation (in mm). As an antifungal positive control, ketoconazole (40µl) was utilized. The Means were computed for each experiment, which was carried out in triplicate.

6. ANTIOXIDANT ACTIVITY

In vitro antioxidant activity ofCNL-AgNPs and *C. nurvala* leafwere determined by using catalase activity, peroxidase activity (enzymatic) and FRAP (non-enzymatic) assay.

Catalase (CAT) activity

With slight modifications, Teranishi*et al.,* 1974 technique's was used to measure catalase activity. A 1 gm sample was homogenized in chilled/ice cold phosphate buffer (50 mM; pH 7.0), and the supernatant was used as an enzyme extract after centrifuging at 10,000 rpm for 10 min at 4 degree C. The reaction mixture (3 ml) is made up of 0.1 ml of supernatant and 2.7 ml of PO_4buffer (50 mM; pH 7.0). H_2O2 (20 mM) was added in 0.2 ml to begin the reaction. For three minutes, at 410nm there absorbance was found to be decreased. The CAT activity was given as gm fw/sec/M H_2O_2 decrease.

Peroxidase activity

With the adjustments listed below, the Chance and Maehly, 1955 technique was used to measure the peroxidase activity. After homogenizing the 0.2gm sample with 10ml of phosphate buffer for 20 minutes, it was centrifuged at 10,000 rpm. The enzyme extract was obtained from the supernatant. Pyrogallol, H_2O_2, and 2.2 ml of phosphate buffer were also added. After adding 0.2 ml of enzyme extract, at 420 nm the absorbance was measured to assess how much purpurogallin had generated.

FRAP assay

Using the Pulido *et al.,* 2000 technique, ferric reducing antioxidant power (FRAP) tests were carried out. After adding 50 ml of methanol, the 5 gm sample was incubated for 24h at 37 °C. Sample from the incubator was filtered and dried in a petri dish. In accordance with 1 mg/ml, the dried material was dissolved in methanol. For FRAP estimate, the sample size range of 10 µl to

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100 µl was chosen.Up to 1 ml of volume was made up with methanol. Prior to use, the FRAP reagent was incubated for 30 minutes at 37°C in 10 ml of acetate buffer (0.2 M; pH 3.6), TPTZ (10 mM) in 1 ml of HCl(40 mM), and 1 ml of $FeCl_3$ (20 mM). Each tube received 1 ml of FRAP reagent, which was carefully vortexed before being tentatively incubated for 30 minutes at 37°C. At 593 nm, absorbance was observed. The blank was made without extracting any material. Micro molars are used to express the results.

RESULTS AND DISCUSSION

1) CHARACTERIZATION OF SILVER NANO-PARTICLES

Scanning electron microscopy (SEM)

Using SEM, the morphology of CNL-AgNPs has been described. Figure 2 displays SEM images of AgNPs that were created by reducing $AgNO_3$ with a leaf extract of *Crataeva nurvala*. After reduction, the colour of CNL-AgNPs changed from brown to dark brown, as shown in Figure 1. AgNPs had a spherical form and ranged in size from 29 to 82 nm. The aqueous leaf extract of *C. spinosa* was used to produce spherical AgNPs in the Salmen*et al.,* 2021 investigation. The nanoparticles' diameters ranged from 5 to 27 nm. Aqueous extract of *Buchholzia coriacea* seed produced AgNPs with a spherical form and sizes ranging from 15 to 50 nanometer were also discovered by Adelere*et al.* in 2017.

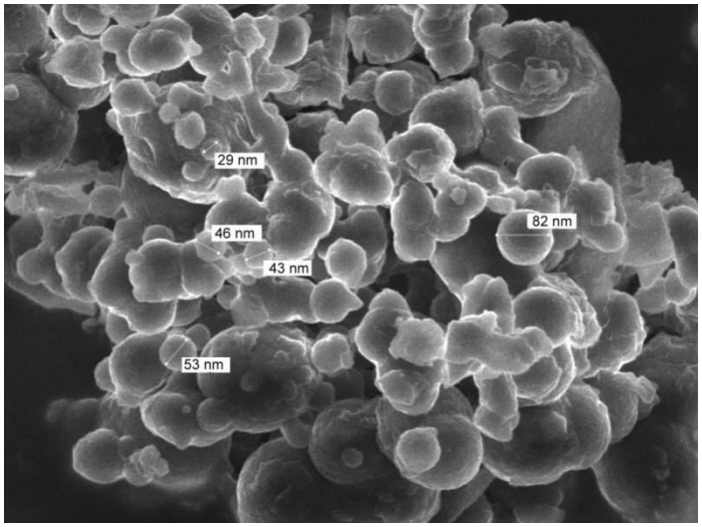

Figure 2: Scanning electron micrograph (SEM) of CNL-AgNPs

Fourier Transform Infrared (FTIR) Spectroscopy

The functional groups of the active bio-molecules acting as reducing and capping agents in the creation of nano-particles were identified using FTIR analysis of the generated CNL-AgNPs. Figure 3 shows the FTIR spectra of CNL-AgNPs. Notable transmittance bands were found at 3435 cm^{-1} (>N-H-), 1689 cm^{-1} (Quinone or conjugated ketone), and 1083 cm^{-1} (Si-O-Si). There

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were also detected at 2928 cm^{-1} (C-H asym./sym.), 1538 cm^{-1} (Aromatic nitro compounds), 1386 cm^{-1} (Organic sulphates), and 688 cm^{-1} in a limited range (C-S-).Sharma *et al.,* 2019 characterized AgNPs synthesized by *A. paniculata*leaf in which prominent transmittance bands were found at 1075 cm^{-1} (-C-N-), 3240 cm^{-1} (-O-H- or -N-H-), 1575 cm^{-1} (-NH-), and 2929 cm^{-1} (-C-H-). In 2021, Salmen*et al.*revealed FTIR spectra of AgNPs synthesized by *C. spinosa* aq. leaf extractwith a broad transmission peak at 2923.81cm^{-1} (alkane stretching band),1109.44cm^{-1} (C-O), 3412.06 cm^{-1} ((H and O-H stretch),617.40cm^{-1} (C=N stretch, and 1633.34cm^{-1} (C=C stretch). Shaikhaldein*et al.,* 2022 reported FTIR profile of AgNPs synthesized by *Maeruaoblongifolia*shoots extract in which five peaks at3435 cm^{-1},1634 cm^{-1},2078 cm^{-1},675 cm^{-1}, and 794 cm^{-1}were found that represent functional groups of various compounds.

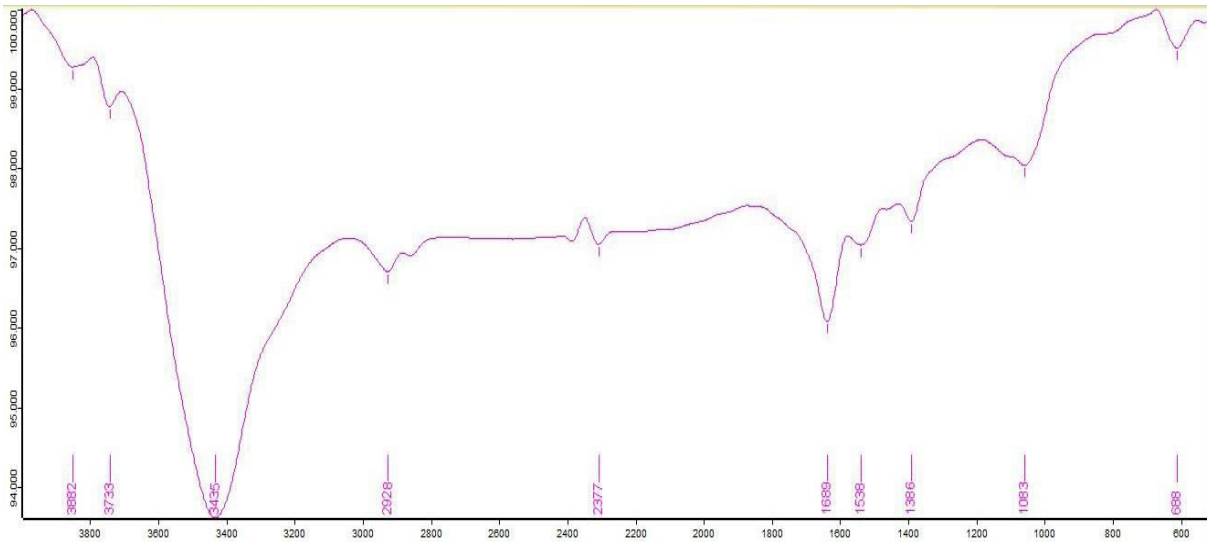

Figure 3: FTIR spectrum of CNL-AgNPs

2) ANTIMICROBIAL ACTIVITY

Antibacterial activity

The AgNPs that were created have strong anti-bacterial properties. According to Khatune *et al.* (2015), the alkaloids, flavonoids, reducing sugar, saponins, steroids, and tannin components included in *C. nurvala* leaf extracts are what give them their medicinal potential as an antioxidant, anthelmintic, antibacterial, and other treatment. In the current study, the antibacterial activity of plant-AgNPs and CNLM was assessed in vitro against four bacterial strains, including gram positive *Staphylococcus aureus*, gram negative *Escherichia coli*, and gram positive *Pseudomonas aeruginosa* bacterial colonies as shown in Table 1 to 4.When methanolic solutions of *C. nurvala* leaf and synthetic silver nanoparticles were compared for antibacterial activity, the maximum zone of inhibition against *S. aureus* was recorded in the plant-AgNPs at 12 mm at (80

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µl), while there was no zone of inhibition recorded for the methanolic leaf extract. *S. aureus* shown resistance to CNLM. AgNPs exhibited antibacterial activity against *Bacillus subtilis* at all concentrations, with the maximum inhibitory activity (15 mm) observed in 80 µl, but in the methanolic extract, activity was only detected in 80 µl with an 8 mm zone of inhibition. Methanolic extract is more efficient against *E. coli* than manufactured Ag nanoparticles, as indicated in Table 4. In comparison to *C. nurvala* leaf methanolic extract, antibacterial activity against *Pseudomonas aeruginosa* was shown to be more effective in both methanolic and silver nanoparticle extract, although was more inhibited by the latter.Using the well diffusion technique, the root bark of *C. nurvala* was extracted with ethanol, while the stem bark was extracted with chloroform, both of which shown notable antibacterial effectiveness against pathogens (Malini*et al.,* 1995). By using the disc diffusion method, the anti-bacterial activity of *C. nurvala* was assessed in contrast to the 4 human pathogenic bacteria like *Escherichia coli*, *Shigelladysenteriae*, *Shigellasonnei*, and *Shigellaboydii* and the two Gram positive such as *Bacillus cereus* and *Bacillus megaterium* and Gram negative bacteria like *Bacillus cereus* and *Bacillus megaterium*). Zones of inhibition for chloroform extract ranged from 8.3 to 22.1 mm at 500 g. *Bacillus megaterium* was not harmed by the extract (Parvin*et al.,* 2012). The ethanolic extract of *Crataevanurvala* leaf did not show any antibacterial action against *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*,*Salmonella typhi*,*Shigella dysenteriae,* and*Escherichia coli*in Khatune *et al*., 2015 investigations.The bacterial strains were fully inhibited by the ethanolic extract of *C. nurvala*. While the present study's methanolic extract demonstrated inhibitory efficacy against*E. coli, B. subtilis*, and *P. aeruginosa*in a range of 8mm to 15mm, manufactured AgNPs of *Crataevanurvala*leaf had more notable antibacterial outcomes relative to the bacterial strains. AgNPs' precise antibacterial mechanisms are yet unclear. However, other researchers have hypothesised that AgNPs' capacity to enter cells may be what causes them to work against bacteria (Sondi and Salopek-Sondi, 2004).In the Loo *et al.,* 2018 study, the green produced AgNPs were able to overwhelm the high conc. of bacteria (about 106 CFU/mL), showing that silver nanoparticle may be employed as a great antibacterial agent.

Antifungal activity

Due to their potent biocidal impact against germs, silver nanoparticles are widely recognized as the most effective antimicrobial agents. They have been used for decades to both prevent and cure a variety of illnesses (Oei*et al.,* 2012). According to Kim *et al.* (2009), silver nano-particle are also often employed as anti-inflammatory (Nadworny*et al.,* 2010). In this work, four different fungal isolates, including *C. albicans*, *A. niger*, *T. reesei*, and *P. chrysogenum*, were exposed to CNLM and silver nanoparticles made from this plant.A good antifungal activity have been recognized only in synthesized silver nanoparticles of *C.nurvala* leaf extract against *Candida albicans*and *Aspergillus niger*rest of show little or no zone of inhibition. The results were represented in Table- 5-8.Khatun*et al.,* (2015) assessed the anti-microbial, anti-oxidant, and phyto-chemical assessment of leaf ethanolic extract of *C. nurvala* and showed the existence of steroids, reducing sugar, tannin, alkaloid, flavonoids, and saponin.

3)ANTIOXIDANT ACTIVITY

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Enzymatic antioxidant activity

Antioxidant enzymes like SOD, CAT, and GPx, which change active oxygen molecules into non-toxic chemicals, are a key part of the body's defensive system. The detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyzes GSH, and the catabolism of H_2O_2 are crucial processes involving CAT and GPx. Protecting ailments after treatment requires antioxidant activity and the prevention of free radical production (Jain *et al.,* 2008).

The well-known antioxidant CAT, which is found in all cells, shields them from the extremely harmful hydroxyl radicals ($OH^{\bullet}$) (Celik*et al.,* 2014). H_2O_2 is created during regular metabolic activities as an unhealthy byproduct. H_2O_2 must be promptly changed into other, less harmful molecules to prevent cell damage. CAT is responsible for doing this task. By turning the H_2O_2 to H_2O and O_2, it neutralizes it (Fernandez *et al.,* 2009). In the current work, *Crataevanurvala*leaf silver nanoparticles had the greatest CAT characteristics (0.062 gm Fw/sec/M H_2O_2 decrease) when compared to methanolic extracts (0.047 gm Fw/sec/M H_2O_2 decrease).

Peroxidase is an oxidoreductase, an enzyme that catalyses the oxidation-reduction reaction that turns various compounds into oxidized by the action of free radicals. Specifically, ferricyanides and ascorbic acid are broken down into harmless components by peroxidase activity by being given electrons (Albuquerque and Rocha, 2019). According to El-Sayed and Verpoorte (2008), peroxidases are the primary enzyme in defense-related pathways in plants and are essential for responding to a wide range of pathogens (Van *et al.,* 2006). They also take part in a variety of fundamental metabolic activities, including the metabolism of auxin, the production of suberin and lignin, the cross-linking of cell wall constituents, the synthesis of phytoalexins, and the metabolism of RNS and ROS (Almagro*et al.,* 2009).In this work, methanolic *C. nurvala* leaf extract shown 0.293 M/L/gm dwt/sec peroxidase activity while silver nanoparticle *C. nurvala* leaf extract demonstrated 0.558 M/L/gm dwt/sec peroxidase activity. As a result, AgNPs had the highest level of peroxidase activity when compared to methanolic extracts.

Non-enzymatic antioxidant activity

Ferric ion reducing antioxidant power (FRAP) Assay

It is well recognized that free radicals play a significant part in a wide range of clinical symptoms. Antioxidants save us from numerous illnesses by battling free radicals. They either work by removing reactive oxygen species from the environment or by defending the antioxidant defence systems (Umamaheswari and Chatterjee, 2008).As similar to CAT and Pox activity, highest Fe^{+3}ion reducing activity was also observed with synthesized silver nanoparticle of *C. nurvala* leaf extract. The antioxidant activity measured by FRAP method show in Table 11, 12. The free radical scavenging activity of the synthesized nanoparticle and methanolic leaf *C. nurvala* extract was found to upsurge with growing concentration as shown in Figure 6-7. At 10 µl conc.ofmethanolic leaf extract of *C. nurvala*119.64 µM µM and at 100 µl 1146.78 µM concentration of ferrous ion was recognized. While CNL-AgNPsexhibited 195.35µM ferrous ions at 10 µl and 1261.07 µM at 100 µl that was maximum with respect to methanolic leaf extract of activity.The antioxidant activity of bioactive substances is related to their growth of

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reducing power and rises with an increase in poly-phenol content (Hu *et al*., 2000; Siddhuraju*et al*., 2002).The radical chain reaction is stopped by phytophenolics, which convert free radicals into more stable byproducts. When poly-phenols such as ascorbic acid,tannic acid,quercetin, caffeic acid, and ferulic acidreact with iron (TPTZ)2 (III), the process is interrupted. This is the appropriate chemical reaction of the FRAP method, which includes a single e^- reaction between iron $(TPTZ)_2$ (III) and a single e^- donor ArOH. The significant anti-oxidative action of the plant leaf against in vitro chemically encouraged free radical production and LPO is the basis for the therapeutic benefits of the leaf (Behera and Senapati, 2014).Antioxidants in the samples would cause Fe^{3+} to be reduced to Fe^{2+} by giving an electron in the reducing power test. The measurement ofFe^{3+} reducing activity made use of the capacity of -carotene and its decomposition products to conduct a single e^- transfer-based reaction (Mueller *et al.,* 2011). The antioxidant test in the *C. nurvala* stem bark reveals that methanolic extract has stronger antioxidant potential than petroleum ether in terms of free radical scavenging capability and ferric reduction capacity (Hade *et al.,* 2016).

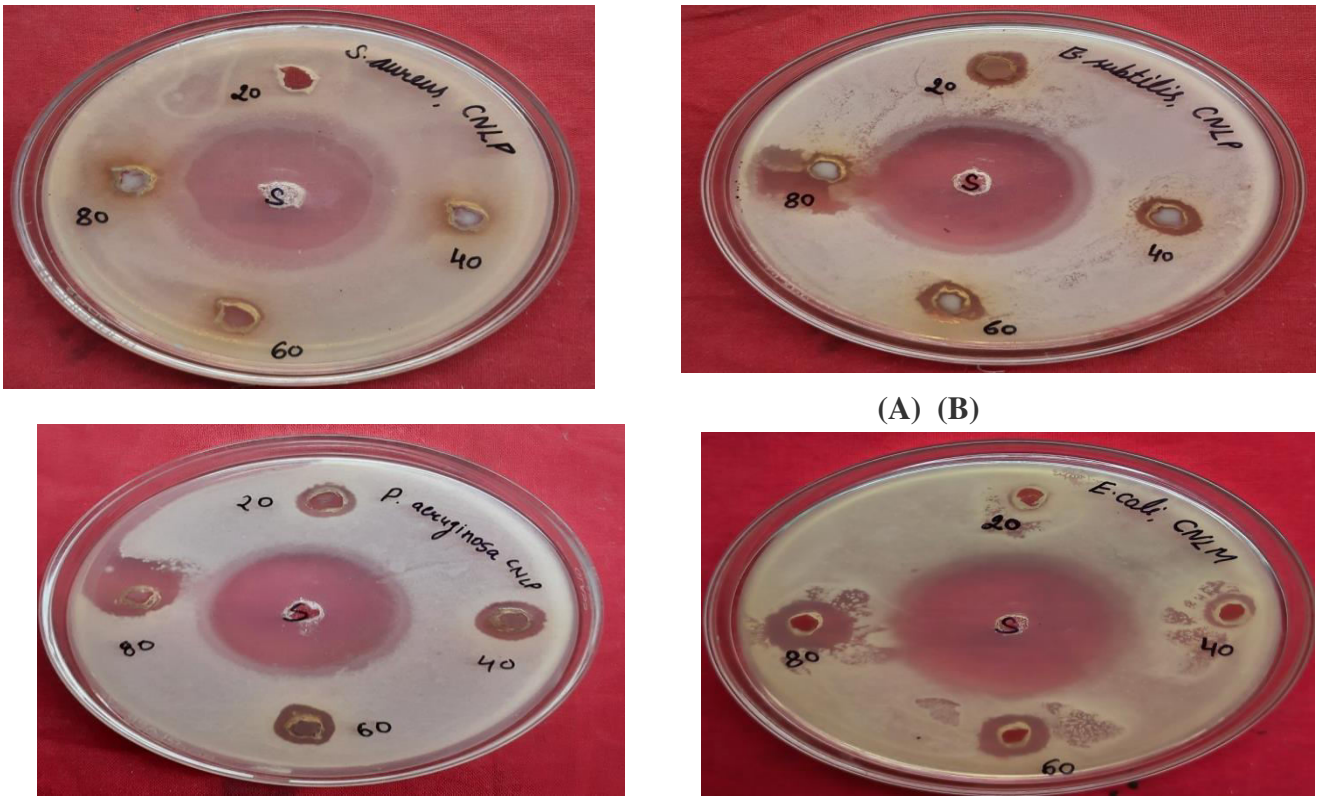

Figure 4: Maximum anti-bacterial activity of synthesized silver nanoparticle (CNLP) and *C.nurvala* leaf methanolic (CNLM) extract against (A) *S. aureus* (B) *B. subtilis* (C) *P. aeruginosa* and (D) *E. coli*

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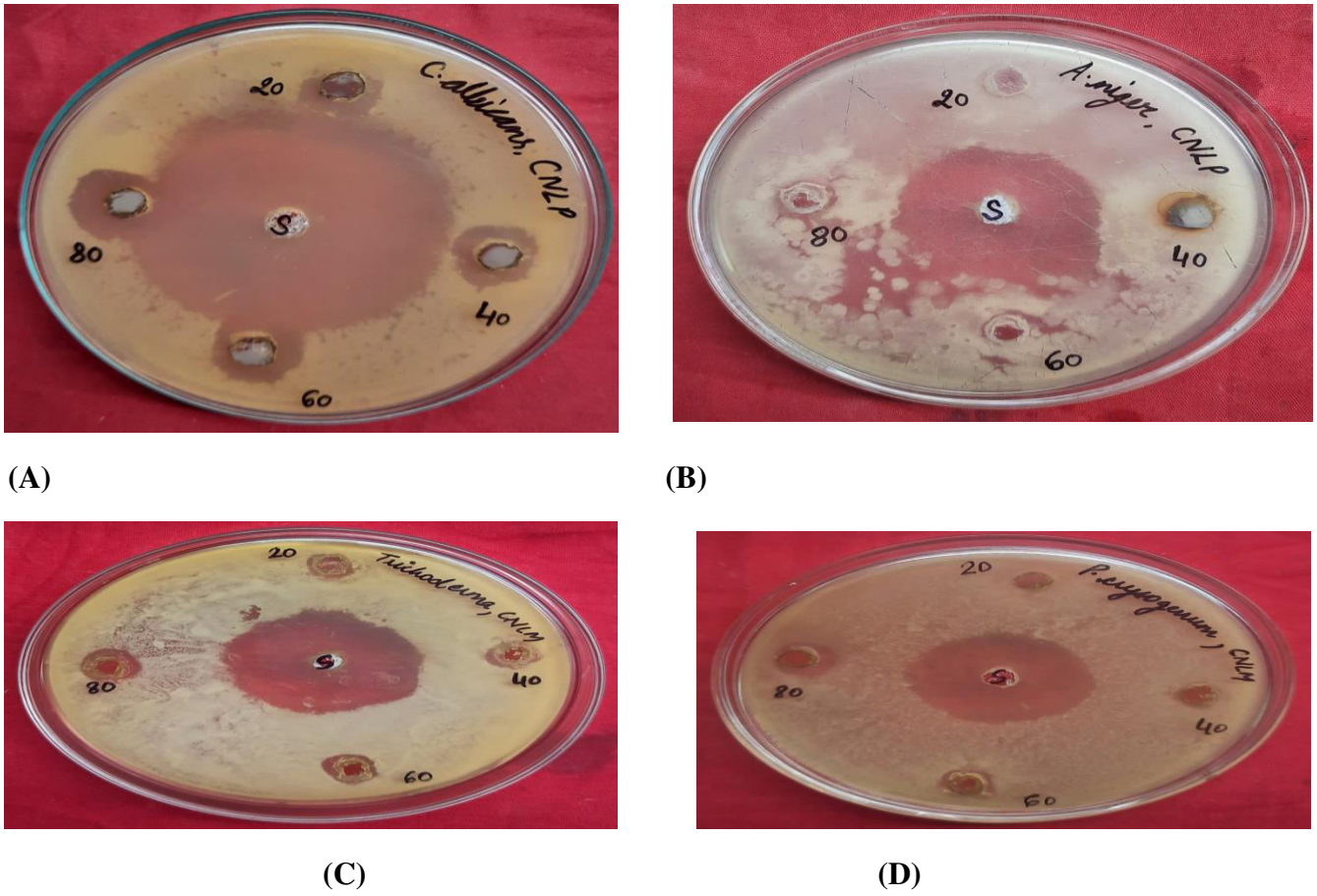

(A) **(B)**

(C) **(D)**

Figure 5: Maximum anti-fungal activity of synthesized silver nanoparticle (CNLP) and *C. nurvala* leaf methanolic (CNLM) extract (A) *C. albicans* (B) *A. niger* (C) *T. reesei*and (D) *P. chrysogenum*

Table 1: Anti-bacterial activity of C*rataevanurvala* leafmethanolic(CNLM) extractand synthesized nanoparticles (CNL-AgNPs)against *S. aureus*

Plant samples	Inhibition zone (mm)				
	Standard	20µl	40µl	60µl	80µl
CNLM	35	Nil	Nil	Nil	Nil
CNL-AgNPs	35	Nil	10	11	12

Table 2: Anti-bacterial activity of against C*rataevanurvala* leafmethanolic (CNLM) extractand synthesized nanoparticles (CNL-AgNPs)against *B. subtilis*

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Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	35	Nil	Nil	Nil	8
CNL-AgNPs	35	11	12	13	15

Table 3: Anti-bacterial activity of *Crataevanurvala*leafmethanolic (CNLM) extract and synthesized nanoparticles (CNL-AgNPs)against *P. aeruginosa*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	35	8	9	11	13
CNL-AgNPs	35	10	11	13	16

Table 4: Anti-bacterial activity of *Crataevanurvala*leafmethanolic (CNLM) extract and synthesized nanoparticles (CNL-AgNPs)against *E. coli*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	35	8	10	13	15
CNL-AgNPs	35	10	11	12	13

Table 5: Anti-fungal activity of *Crataevanurvala* leaf methanolic(CNLM) extract and synthesized nanoparticles (CNL-AgNPs) against *C. albicans*

Plant samples	Inhibition zone (mm)				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**

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CNLM	32	Nil	8	9	10
CNL-AgNPs	32	13	14	15	16

Table 6: Antifungal activity of *Crataevanurvala* leaf methanolic(CNLM) extract and silver nanoparticles (CNL-AgNPs) against *A. niger*

Plant samples	**Inhibition zone (mm)**				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	32	8	9	10	11
CNL-AgNPs	32	13	14	15	16

Table 7: Antifungal activity of *Crataevanurvala* leaf methanolic(CNLM) extract and silver nanoparticles (CNL-AgNPs) against *T. reesei*

Plant samples	**Inhibition zone (mm)**				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	32	9	10	11	13
CNL-AgNPs	32	Nil	9	11	13

Table 8: Antifungal activity of C*rataevanurvala* leafmethanolic (CNLM) extract and silver nanoparticles (CNL-AgNPs) against*P. chrysogenum*

Plant samples	**Inhibition zone (mm)**				
	Standard	**20µl**	**40µl**	**60µl**	**80µl**
CNLM	32	Nil	9	11	12
CNL-AgNPs	32	Nil	Nil	Nil	Nil

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Table 9: Catalase activity of *Crataevanurvala*and synthesized nanoparticles

Plant Samples	Catalase activity (gm Fw/sec/M H_2O_2decrease)
*C.nurvala*leaf	0.047±0.005
CNL-AgNPs	0.062±0.007

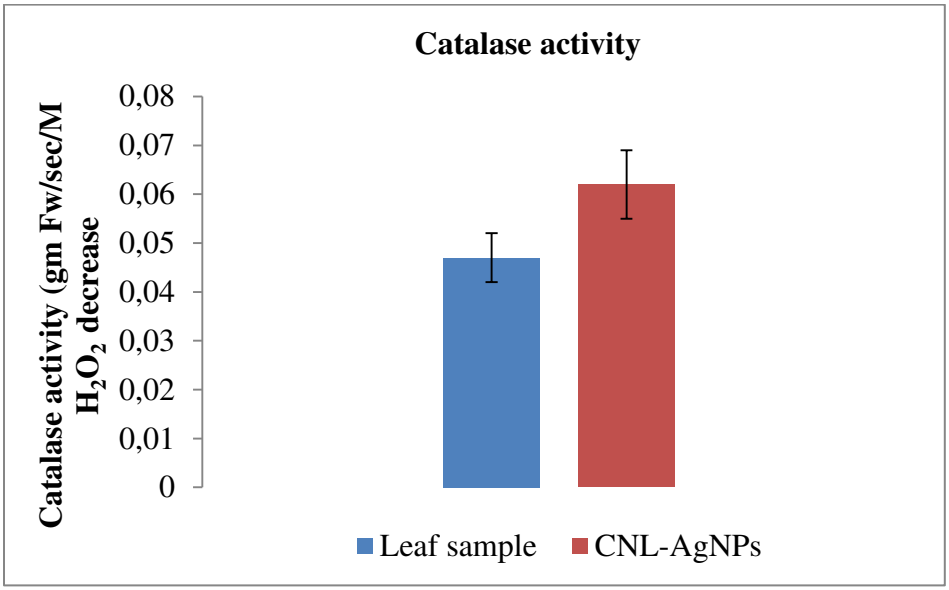

Figure 5: Catalase activity of C*rataevanurvala*leaf extract and silver nanoparticle

Table 10: Peroxidase activity in of *Crataevanurvala*and synthesized nanoparticles

Plant Samples	Peroxidase activity (µM/L/gm dw/sec.)
*C.nurvala*leaf	0.293±0.02
CNL-AgNPs	0.558±0.06

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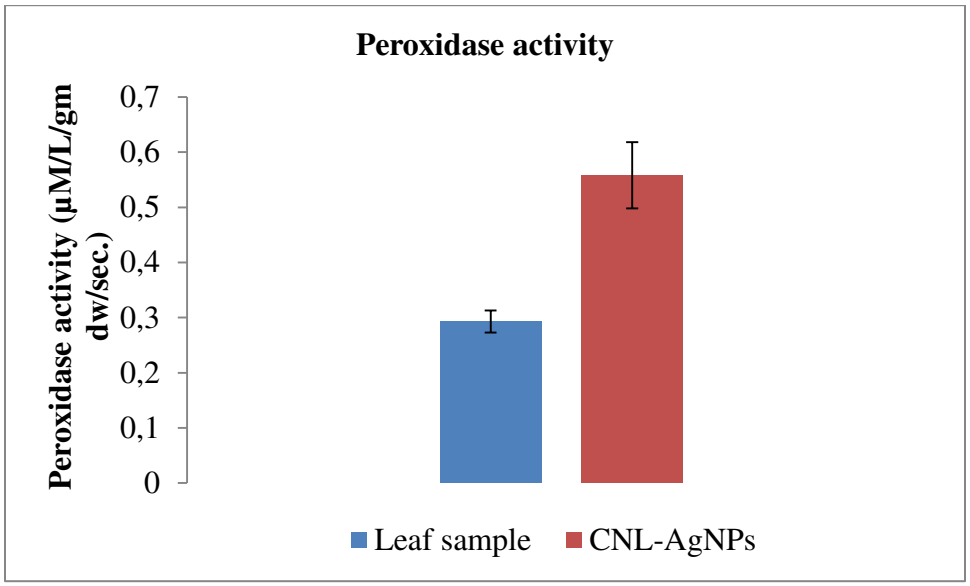

Figure 6: Peroxidase activity C*rataevanurvala*leaf extract and silver nanoparticle

Table 11: FRAP activity of *Crataevanurvala* leaf

***C. nurvala*leaf sample**	**Concentration (µM)**
10 µl	119.64±4.91
20 µl	219.64±1.03
30 µl	351.07±3.34
40 µl	504.64±1.67
50 µl	618.21±5.75
60 µl	788.21±0.97
70 µl	846.78±2.06
80 µl	993.21±4.11
90 µl	1078.21±2.18
100 µl	1146.78±5.08

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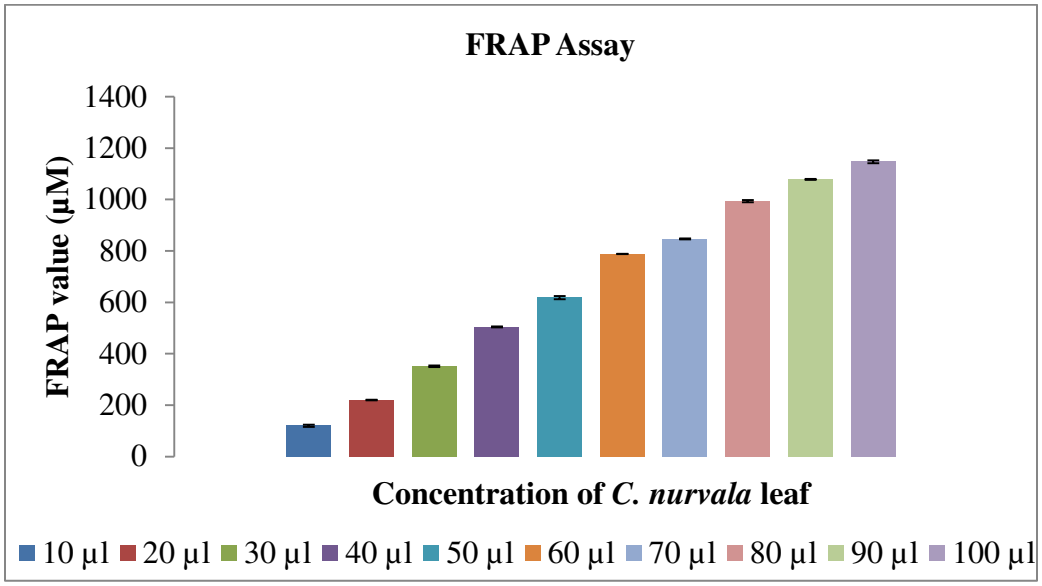

Figure 6: FRAP assay C*rataevanurvala*leaf extract

Table 11: FRAP activity of CNL-AgNPs

CNL-AgNPs	Concentration (µM)
10 µl	195.35±2.89
20 µl	310.35±1.67
30 µl	412.5±0.57
40 µl	493.92±4.99
50 µl	626.07±5.78
60 µl	898.92±3.42
70 µl	944.64±1.13
80 µl	1048.21±2.06
90 µl	1174.64±3.11
100 µl	1261.07±4.37

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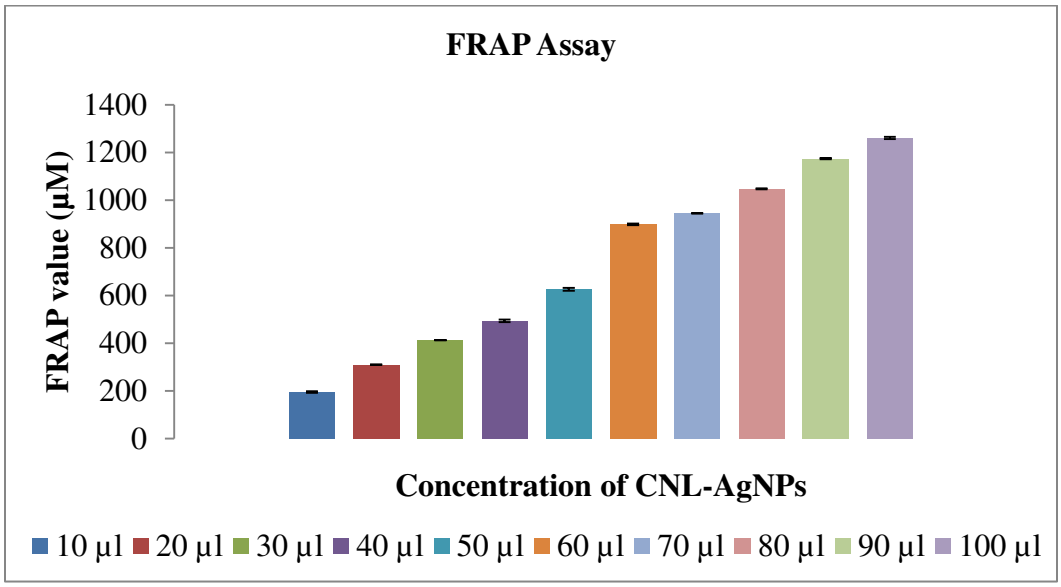

Figure 7: FRAP assay of CNL-AgNPs

CONCLUSION

According to this study, *Crataevanurvala*leaf has less capacity to suppress pathogens and scavenge free radicals than silver nanoparticles made by this plant. Due to the fact that AgNPs can change the assembly of cell membranes by pervading into cell wall of bacteria. Due to huge surface area to volume ratio and their nano-scale size which determines its efficiency. By dischargingAg^{+}, they can make cell membranes more permeable and generate reactive oxygen species. Because silver nanoparticles include bioactive chemicals on their surface, they also exhibit potent antibacterial and antifungal properties. Therefore, because they are non-toxic, affordable, eco-friendly, and extremely powerful against bacteria, silver nanoparticles made by green synthesis may be employed as antibiotics in the future.

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